THE CANADIAN VETERINARY JOURNAL
LA REVUE VÉTÉRINAIRE CANADIENNE
Volume 13 May-mai 1972 No. 5

SERUM IRON LEVELS IN NORMAL AND ANEMIC HORSES

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Introduction

When reviewing the literature on iron metabolism in the horse, Kolb (6) emphasized the disparity in the reported normal serum values. The present paper reports a study of the effect of age, sex, breed and diurnal variation on serum iron concentration and total iron binding capacity (TIBC). Also reported is the alteration in serum iron concentration in anemia produced by (a) experimental equine infectious anemia (EIA), (b) hemolysis due to phenothiazine administration, and (c) repeated moderate hemorrhage.

Materials and Methods

Normal horses: The population distribution of serum iron concentration and TIBC was determined in 18 Thoroughbred horses, 33 Quarter horses, three Thoroughbred X Quarter horses, 14 Arabian horses, two Paint, two Appalloosa, two Welsh, two American Saddle, one Tennessee Walking horse and eight Shetland ponies. The animals were between one and 16 years old. The same parameters were measured daily in four Standardbred horses which had been stabled for several months. To minimize the effects of diurnal variation blood samples from these four horses were withdrawn at approximately 9 a.m. each day. All horses were clinically normal. They were fed 1.5 kg of oats/100 kg bodyweight between 8 and 9 a.m. and a generous quantity of good quality prairie hay after 2 p.m. Water was provided *ad libitum*.

Diurnal variation in serum iron concentration was studied in three healthy Shetland ponies. They were fed the standard ration of oats between 8 and 9 a.m. and hay after 2 p.m. Blood samples for serum iron determination were taken approximately every three hours for 88 hours. Total iron binding capacity of serum was measured once each day.

Anemic horses: Experimental EIA was produced in three Shetland ponies by whole blood inoculation from an infected animal. The ponies were bled daily for 60 days after injection. They were then killed and EIA confirmed at necropsy. A more extensive report of this experiment has been published (10).

Non-infectious hemolytic anemia was produced in four Shetland ponies by administering 20 gms of phenothiazine orally. Blood samples were withdrawn daily from each of these animals on three days before dosing and for 20 days after dosing.

Experimental acute hemorrhage was produced by bleeding three horses of 12½% of the calculated blood volume on each of days 1, 3, 7, 10, 14, 17, 20 and 24. Blood volume was considered to be 75 ml/kg bodyweight. Blood samples were taken before and occasionally in the interval between each phlebotomy.

For serum iron and TIBC determinations, 10 ml blood was withdrawn from the jugular vein into plastic sterile syringes using an 18 gauge needle. After two to three hours standing at room temperature, serum was separated by centrifugation at 2500 rpm for ten to 20 minutes and then stored at 4° C until analyzed. Iron concentration and TIBC were measured by standard procedure (4). All determinations were made using an Hitachi Coleman 101 spectrophotometer with a 100 mm light path. Included with each batch of specimens was a control iron solution containing 200 μg/100 ml. Mean value of the optical density of this standard was 0.191, range 0.185–0.193 in the entire study. To test the reproducibility of the procedure, 12 serum samples were analyzed before and after frozen storage for one month.

Results

The reproducibility of the analytical procedure was evaluated. Twelve serum samples were kept in frozen storage for one month. The serum iron concentration was determined be-

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TABLE I

SERUM IRON CONCENTRATION AND TIBC OF NORMAL HORSES, VARIATION DUE TO BREED, AGE AND SEX

	Number Animals	Serum[1] Iron Concentration μg/100 ml	TIBC[1] μg/100 ml
A. *Breed*			
Arab	14	129±29	189±17
Quarter Horse	33	154±34	337±49
Shetland	8	106±31	344±62
Thoroughbred	18	109±12	297±47
Others	12	110±26	246±29
B. *Age*			
Under 5 years	36	134±32	294±47
6–9 years	23	124±35	281±60
Over 10 years	21	130±29	303±38
C. *Sex*			
Male	39	128±28	298±26
Female	42	132±34	282±48

[1]mean ± S.D.

fore and after storage. The mean concentration of the 24 determinations was 80.5 μg/100 ml and the standard error of deviation was 1.48 μg/100 ml.

Table I shows the age, breed and sex variations in serum iron concentration and TIBC in 85 normal horses. The three crossbred horses, two Paint, two Appalloosa, two Welsh, two American Saddle and one Tennessee Walking horse have been included in this table under the heading of "others." Mean value of serum iron concentration in the total population was 130 ± 27 μg/100 ml. The 33 Quarter horses had higher serum iron concentrations, 154 ± 34 μg/100 ml than did the other breeds. Mean coefficient of variation of serum iron concentration for the five breed groups was 21.6%. The TIBC of the serum of the Arabian horses, 189 ± 17 μg/100 ml, was significantly lower ($P < 0.01$) than that of the other horses. Horses included in the grouping "others" had significantly lower TIBC of the serum, 246 ± 29 μg/100 ml, ($P < 0.05$) than Quarter horses, Thoroughbreds or Shetland ponies which had concentrations of 337 ± 49 μg/100 ml, 297 ± 47 μg/100 ml and 344 ± 62 μg/100 ml respectively. No age or sex differences were found in the serum iron concentration or TIBC values.

The relationship of serum iron concentration to TIBC of the serum showed breed differences. The mean saturation of siderophilin in Arabian horses was 68.3%, in Quarter horses 45.7%, in Shetland ponies 30.8%, in Thoroughbred horses 36.7% and in the other breeds 44.7%.

To study the daily variation in serum iron concentration and TIBC, blood samples were withdrawn at the same time each day for 21 days from four stabled Standardbred horses. Mean and range for each value are contained in Table II. These values showed less variation than the random samples from the populations. However, on a daily basis the values were not constant. It is unlikely this daily fluctuation was associated with external factors as these animals had been stabled, fed a standard diet and were accustomed to their surroundings and attendants.

Figure 1 shows the diurnal changes in serum iron concentration in three Shetland ponies. No consistent diurnal pattern was observed. Increased serum iron concentrations always occurred after the hay ration had been fed in the early afternoon. A similar response was not consistently observed following the feeding of the cereal ration. The mean TIBC of the serum in four random blood samples taken from each horse were 432, 316 and 373 μg/100 ml. The corresponding serum iron concentrations were 187, 127 and 133 μg/100 ml and siderophilin saturations were 43.3%, 40.1% and 35.6%. In each horse, hematocrit showed small variations (± 2% of the initial packed cell volume).

Figure 2 shows sequential changes in serum iron levels in horses with anemia caused by acute EIA, phenothiazine toxicity and repeated exsanguination. The initial response to each anemia was an increased serum iron concentration. In the horses subjected to repeated phlebotomy this initial response did not persist and low serum iron concentration became pronounced although these animals continued in good appetite and showed no clinical reaction

TABLE II

MEAN AND RANGE OF SERUM IRON CONCENTRATION AND TIBC IN 21 CONSECUTIVE DAILY BLOOD SAMPLES FROM FOUR HORSES

Horse	Serum Iron Concentration μg/100 ml	TIBC of Serum μg/100 ml	% Saturation of Siderophilin
1	193 (165–234)	306 (282–345)	63 (55–71)
2	235 (190–282)	380 (350–435)	62 (48–72)
2	219 (200–258)	271 (240–300)	81 (65–91)
4	153 (106–188)	258 (240–282)	59 (44–67)

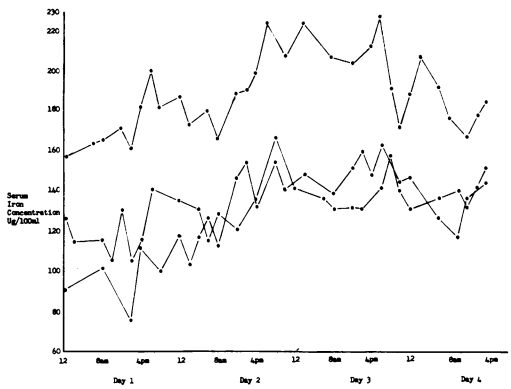

FIGURE 1. Diurnal variation in serum iron concentration in three normal horses.

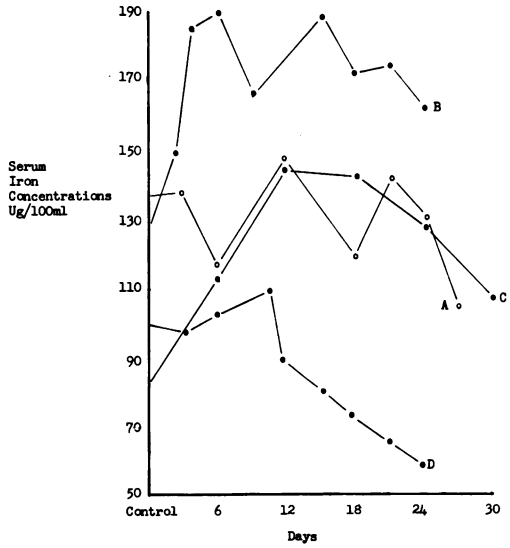

FIGURE 2. Serum iron concentration in normal and anemic horses: A–(open circles) normal horses, B–horses with phenothiazine toxicity, C–EIA affected horses, D–horses undergoing repeated hemorrhage.

to the experimental procedure. The horses with EIA did not develop low serum iron concentrations even though they became quite weak and lost considerable body weight.

DISCUSSION

The serum iron concentrations in Thoroughbreds and Shetland ponies (108μg/100 ml) are similar to reported values for other normal horse populations (6, 9, 10). Seckington *et al* (13) reported mean serum iron concentration of six recently foaled, aged, grass fed mares to be 107μg/100 ml (range 58–191 μg/100 ml). In that study, ten grass fed, barren mares were found to have mean serum iron concentration of 153 μg/100 ml (range 114–193 μg/100 ml); the corresponding values for nine stable fed horses, diet not reported, was 144 μg/100 ml (range 70–216 μg/100 ml). Kolb (6) has reported the TIBC of the serum in eleven healthy horses as 200 μg/100 ml whereas other workers (9, 10, 11) reported TIBC values in the order of 330 μg/100 ml and Seckington *et al* (13) reported 720 μg/100 ml and 592 μg/100 ml for grass fed and stabled horses respectively.

The Shetland ponies had significantly lower percentage saturation of siderophilin as compared with all breeds except the Thoroughbred horses. In contrast, the Arabian horses had significantly greater percentage saturation of siderophilin. Decreased amounts of siderophilin in plasma of man have been reported in hemolytic anemia (1). Increased percentage saturation occurs in hemochromatosis (2). Neither of these conditions was apparent in the Arabian horses. It has been shown that there are several siderophilin moieties in horses, each under individual genetic control (3). One possible explanation for the low siderophilin levels in Arabian horse plasma may be genetically determined shorter half-life of this protein.

The data in Figure 1 suggest that feeding

may have an important influence on serum iron concentration, adding confirmation to the work of Schutzler and Wiedner (12). Factors known to affect the rate of iron absorption are rate of erythropoiesis (8) and level of saturation of siderophilin (5). In this present experiment, hematocrit remained constant, and TIBC and percentage saturation of siderophilin were normal. Thus increased erythropoiesis or undersaturation of siderophilin were not likely to exist in these horses. Figures 1 and 2 show that except in extreme conditions, serum iron concentration has limited value for clinical interpretation.

Summary

Serum iron concentration and total iron binding capacity (TIBC) were determined in 85 unrelated horses, one to 16 years old. Mean serum iron concentration was 129, 154, 106, and 109 μg/100 ml and mean TIBC was 189, 337, 344, and 297 μg/100 ml for Arabian horses, Quarter horses, Shetland ponies and Thoroughbred horses, respectively. No age or sex differences were found. As normal ranges for these parameters are so large, individual serum iron or TIBC determinations have little clinical value.

Serum iron concentration was labile and increased after feeding. The initial response to anemia due to infection, toxicity or repeated blood loss was increased serum iron concentration. Only in those horses subjected to repeated hemorrhage was there a marked decrease in serum iron levels following the initial rise.

Résumé

Cette étude visait à déterminer la teneur en fer du sérum ainsi que la capacité totale de ce dernier à le fixer (TIBC), chez 85 chevaux âgés de un à 16 ans et ne possédant aucun lien de parenté. La concentration moyenne du sérum en fer était de 129, 154, 106 et 109 μg/100 ml, tandis que la moyenne de la TIBC était de 189, 337, 344 et 297 μg/100 ml, selon qu'il s'agissait de chevaux arabes, de "Quarter horses," de poneys Shetland ou de chevaux Thoroughbred, indépendamment de l'âge ou du sexe. Comme ces paramètres comportent normalement de grandes variations, la valeur clinique des déterminations individuelles du fer sérique ou de la TIBC demeure négligeable.

La concentration du sérum en fer s'avérait instable et augmentait après les repas. La réponse initiale à une anémie consécutive à une infection, à une intoxication ou à des pertes de sang répétées consistait en une augmentation de la teneur en fer du sérum. Seuls les chevaux soumis à des saignées répétées subissaient une diminution appréciable de la teneur du sérum en fer, consécutivement à l'augmentation initiale.

References

1. Awai, M. and E. B. Brown. Studies on the metabolism of human transferrin. J. lab. clin. Med. 58: 797–803. 1961.
2. Finch, S. C. and C. A. Finch. Idiopathic hemochromatosis, an iron storage disease. Med. 34: 381–397. 1955.
3. Gahne, B. Studies on the inheritance of electrophoretic forms of transferrins, albumins, prealbumins and plasma esterases in horses. Genetics 53: 681–684. 1966.
4. Goodwin, J. F., B. Murphy and M. Guillemette. Direct measurement of serum iron and iron binding capacity. Clin. Chem. 12: 47–51. 1966.
5. Hallberg, L. and L. Sowell. Iron absorption studies. Acta med. Scand. 168: 3–22. 1960.
6. Kolb, E. Iron metabolism in farm animals. *In* Advances in Veterinary Science. C. A. Brandley and E. L. Jungherr, eds. pp. 58–114. New York: Academic Press. 1963.
7. Kolb, E. Die eisenbindungskapazitat in serum von haustieren. Proc. 16th International Vet. Cong. 2: 31–35. 1959.
8. Moore, C. V. The importance of nutritional factors in the pathogenesis of iron deficiency anemia. Scand. J. clin. lab. Invest. 9: 292–298. 1957.
9. Obara, J., H. Yamamoto and H. Nakajima. Iron metabolism in equine infectious anemia. Bull. Natn. Inst. Anim. Health, Tokyo. 33: 103–110. 1957.
10. Osbaldiston, G. W., J. R. Coffman, S. M. Kruckenberg and H. W. Leipold. Biochemical alterations in equine infectious anemia. J. comp. lab. Med. 3: 37–44. 1969.
11. Planas, J. and S. de Castro. Serum iron and total iron binding capacity in certain animals. Nature, Lond. 187: 1126–1127. 1960.
12. Schultzer, G. and W. Wiedner. Der serumeisenspiegel geounder pferde. Menatsh. Veterinarmend. 11: 265–271. 1956.
13. Seekington, I. M., R. G. Huntsman and G. C. Jenkins. Serum folic acid levels in grass fed and stabled horses. Vet. Rec. 81: 158–160. 1967.